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Original article

Comparison of low level laser, ultrasonic therapy and association in joint pain in Wistar rats

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ABSTRACT

Introduction: Both therapeutic ultrasound as a low level laser therapy are used to control musculoskeletal pain, despite controversy about its effects, yet the literature is poor and also presents conflicting results on possible cumulative effects of techniques association. The aim was to compare the antinociceptive effects of low level laser therapy, therapeutic ultrasound and the association.

Methods: 24 Wistar rats were divided into: GPL – induction of hyperesthesia in the right knee, and untreated; GUS – treated with therapeutic ultrasound (1 MHz, 0.4 W / cm²) GL – low intensity laser (830 nm, 8 J/cm²); GL+US – treated with both techniques. To produce the hyperesthesia 100 µl of 5% formalin solution were injected into the tibiofemoral joint space, which was assessed by von Frey filament digital before (EV1), 15 (EV2), 30 (EV3) and 60 (EV4) minutes after induction.

Results: In comparison within groups, for the withdrawal threshold when the filament was applied to the knee, the back to baseline was observed only for GUS. Comparisons between groups were not different in EV3, and GL was higher than GPL. In EV4 the three groups effectively treated were higher than placebo. On withdrawal threshold on the plantar surface, GL showed return to baseline values already in EV3, and GUS and GL+US returned in EV4. Comparing the groups in EV3 there was a significantly lower threshold to compare GPL with GL and GUS ($p < 0.05$), and there was only EV4 differences when comparing GPL with GUS.

Conclusion: Both modalities showed antinociceptive effects.

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Comparação entre o laser de baixa potência, ultrassom terapêutico e associação, na dor articular em ratos Wistar

R E S U M O

Palavras-chave:

Medição da dor

Terapia a laser de baixa intensidade

Terapia por ultrassom

Introdução: Tanto o ultrassom terapêutico quanto o laser de baixa potência são utilizados para o controle da dor musculoesquelética, apesar de controvérsias. Ainda, a literatura é pobre e também apresenta resultados controversos sobre efeitos cumulativos da associação de técnicas. Assim, o objetivo foi comparar os efeitos antinociceptivos do laser, do ultrassom e da associação destes.

Métodos: Foram utilizadas 24 ratas, divididas em: GPL – indução de hiperestesia no joelho direito, e não tratadas; GUS – ultrassom terapêutico (1 MHz, 0,4 W/cm²); GL – laser de baixa potência (830 nm, 8 J/cm²); GL+US – tratadas com as duas técnicas. Para a hiperestesia foram injetados no espaço tibio-femoral 100 µl de solução de formalina 5%, e avaliada por filamento de von Frey digital, antes (AV1), 15 (AV2), 30 (AV3) e 60 (AV4) minutos após a indução.

Resultados: Na comparação dentro dos grupos, para o limiar de retirada quando o filamento foi aplicado nos joelhos, foi possível observar volta aos valores basais apenas para GUS. Nas comparações entre os grupos houve diferenças em AV3, sendo que GL foi maior do que PL. Em AV4 os três grupos tratados apresentaram valores maiores que o placebo. No limiar de retirada na superfície plantar GL mostrou retorno dos valores basais em AV3, e GUS e GL+US retornaram em AV4. Na comparação entre os grupos, em AV3 havia um limiar menor em GPL ao comparar com GL e GUS ($p < 0,05$), e em AV4 só havia diferenças ao comparar GPL com GUS.

Conclusão: Ambas as modalidades apresentaram efeitos antinociceptivos.

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Introduction

In cases of joint pain such as that arising from knee osteoarthritis conditions, low-level laser therapy has been suggested as therapeutic modality,¹ with reports of benefits for both acute and chronic conditions.² The analgesic effect of this modality may occur by reduction of inflammation mediators,³ changes in neurotransmission⁴ and also by release of endogenous endorphins.⁵ However, in cases of deep pain in individuals with acute and chronic low back pain, more expressive effects of low level laser therapy *versus* placebo added to thermotherapy were not observed by Ay, Dogan and Evcik;⁶ and even in cases of peripheral joints, there is no consensus in the literature.⁷

Ultrasound therapy is likely to be the most widely used physical agent in clinical practice, and its effects may occur by increasing the temperature or by the so-called non-thermal agents, via acoustic cavitation and flow.⁸ Ultrasound is also a modality that can promote pain reduction in cases of osteoarthritis.^{9,10} However, some studies show conflicting results regarding its effects, just as it occurs with the low level laser modality.^{11,12}

When one seeks to assess deep articular pain, the model using formalin injection proves to be valid, yielding reliable results with respect to behavioral patterns in experimental animals. This allows an evaluation of the effect of different modalities of therapy that influence behaviors, that is, stimulating or decreasing nociception.¹³ Thus, it is interesting to use this model to compare the effects of isolated and associated therapeutic techniques.¹⁴ For all these reasons, this study aimed to compare the antinociceptive effects of low level laser, therapeutic ultrasound and the combination of these modalities, i.e., the use of the two techniques consecutively on the same animal.

Materials and methods

Experimental groups

Twenty-four female rats of the Wistar strain were used, weighing 296.10 ± 33.25 g. These animals were obtained from the UNIOESTE Central Animal Bioterium, and were kept in polypropylene cages with free access to water and with food *ad libitum*, with light-dark cycle of 12 hours at controlled room temperature ($24 \pm 1^\circ\text{C}$). The study was conducted according to international standards of ethics in animal experimentation and was approved by the UNIOESTE Ethics Committee on Animal Use, under Resolution 03512.

The animals were randomly divided into four groups:

- Group 1 (GPL, $n = 6$) - composed of animals that underwent induction of right knee hyperaesthesia, untreated (i.e., placebo), subject only to contact with the ultrasound transducer and laser pen;
- Group 2 (GUS, $n = 6$) - right knee hyperaesthesia, treated with therapeutic ultrasound;
- Group 3 (GL, $n = 6$) - right knee hyperaesthesia, treated with low-level laser;
- Group 4 (GL+US, $n = 6$) - right knee hyperaesthesia, treated with the two aforementioned techniques.

Hyperaesthesia induction

To induce hyperaesthesia, shaving was performed on the medial aspect of the right knees and then each animal was manually restrained and injected with 100 µl of 5% formalin solution in its tibiofemoral joint space.¹³

Assessment of nociception

For an evaluation of nociception, the digital von Frey filament (Insight®), which tests nociceptive sensitivity to mechanical stimulus in animals, was used.¹⁵ The test was performed with the animal manually restrained, and the filament applied to the medial aspect of the tibiofemoral joint of the right hind limb. The polypropylene filament tip was applied perpendicularly to the area, with a gradual increase in pressure, and as soon as the animal withdrew the member the test was interrupted to record the withdrawal threshold.

Then the animal was placed in a wooden box with acrylic cover, and its bottom was made of metal trelliswork, through which it was possible to insert the filament in the plantar region of the feet of the animal. Again, pressure was applied and gradually increased until the animal withdrew the limb.

The study personnel were trained for five days in the nociceptive testing procedures. In the day following the last training day, values of limb withdrawing were collected before (AV1) and after 15 (AV2) and 30 (AV3) minutes and, finally, after one hour (AV4) of the induction of hyperaesthesia.

Treatment protocols

After the second evaluation, the treatment was initiated, i.e., 15 minutes after induction of hyperalgesia. G1 did not suffer any therapeutic intervention (only simulation).

In G2 and G4, the animals were transcutaneously treated with ultrasound (Ibramed®), frequency = 1 MHz, ERA head = 1 cm², power density = 0.4 W/cm², on the knee joint interline with slow, circular and rhythmic movements.¹⁶

G3 and G4 animals were treated with low-level laser 830 nm (Ibramed®), power output = 30 mW, inner beam area = 0.11600 cm², fluence = 8 J/cm².¹⁷ After the final evaluation, the animals were euthanized by decapitation in guillotine.

Statistical analysis

Data normalcy was checked by using the Kolmogorov-Smirnov test, and in view of its normality, an intragroup analysis was done by ANOVA with one-way repeated measures for comparison between groups. In all cases the adopted level of significance was 5%. For the sample size chosen (six animals per group), with a standard deviation of 25 and a difference to be detected = 35 g for a significance level of 5%, the test power was 80%. The effect size for each variable was calculated considering the strength of the effect as small (*r* value 0.10 to 0.29), medium (*r* value 0.30 to 0.49) and large (value *r* ≥ 0.50).

Results

Withdrawal threshold - knee

For an evaluation of the withdrawal threshold when the filament was applied to the knee, it was noted in GPL a significant reduction of the threshold (*P* < 0.05) (Fig. 1A). In GUS a significant reduction in the threshold was noted for AV2 and AV3, compared to AV1. But for AV4 no difference was observed, and also AV4 was significantly higher than AV2 and AV3 (Fig. 1B).

Both for GL (Fig. 1C) and for GL+US (Fig. 1D), the behavior was similar to that observed in GPL (placebo).

In the comparisons among groups, differences in AV3 and AV4 were noted: in AV3, GL values were significantly higher than PL values (*P* < 0.05). In AV4, the three groups treated effectively showed higher values than placebo (*P* < 0.05), but with no differences among them (*P* > 0.05). In assessing the homogeneity of variances (Levene's test) and the effect size, it was possible to observe in AV1 *P* = 0.144 and 0.48, AV2 *P* = 0.001 and 0.23, AV3 *P* = 0.675 and 0.68, and AV4 *P* = 0.068 and 0.75, respectively.

Withdrawal threshold - plantar area

For an evaluation of the withdrawal threshold when the filament was applied to the plantar surface, the placebo group (GPL) again showed a significant reduction of the threshold (*P* < 0.05) (Fig. 2A). GUS showed a significant reduction in the threshold in AV2 and AV3 versus AV1. However, there was no difference in AV4. This last time point was significantly higher than AV2 (Fig. 2B), with the behavior of GL+US being similar (Fig. 2D). In the case of GL, significant reduction of the threshold was noted only when comparing AV1 with AV2 (Fig. 2C).

In comparisons among groups, again we were able to discern differences for AV3 and AV4. In AV3 a significantly lower threshold for GPL was detected, compared with GL and GUS (*P* < 0.05); in AV4, differences were observed only in the comparison between GPL and GUS, and GUS maintained a higher threshold (*P* < 0.05). Again, there were no differences among treatment groups (*P* > 0.05). In assessing the homogeneity of variances (Levene's test) and the effect size, we observed in

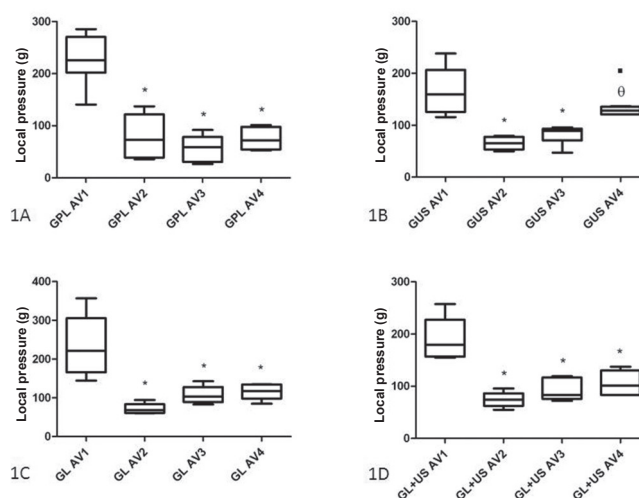


Fig. 1 – Box plot graphics with interquartile intervals, showing the values observed in the evaluations of the different groups with respect to the withdrawal threshold of the limb when the filament was applied to the medial aspect of the knee, at different time points of evaluation (AV1-AV4). 1A - placebo group (GPL), 1B - ultrasound group (GUS), 1C - laser group (GL) and 1D - Laser group associated with ultrasound (GL+US). * Significant difference when comparing with AV1. θ significant difference when comparing with AV2. ■ Significant difference when comparing with AV3.

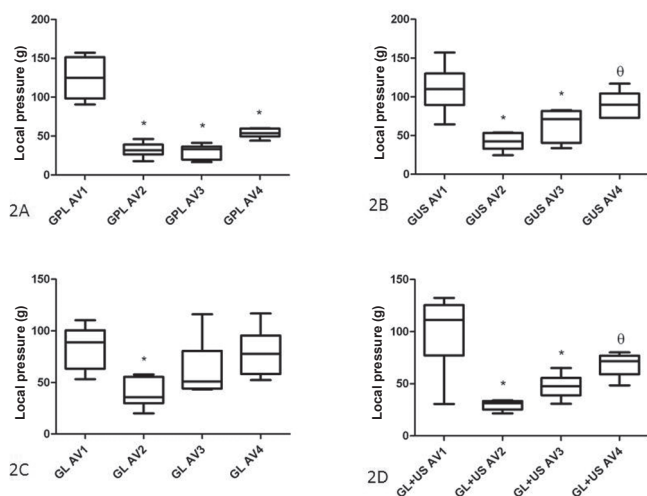


Fig. 2 – Box plot graphics with interquartile intervals, showing the values observed in the evaluations of the different groups with respect to the withdrawal threshold of the limb when the filament was applied to the plantar area, at different time points of evaluation (AV1-AV4). 2A - placebo group (GPL), 2B - ultrasound group (GUS), 2C - laser group (GL) and 2D - laser group associated with ultrasound (GL+US). * Significant difference when comparing with AV1. θ significant difference when comparing with AV2.

AV1 $P = 0.770$ and 0.47 , AV2 $P = 0.138$ and 0.47 , AV3 $P = 0.140$ and 0.62 , and AV4 $P = 0.208$ and 0.69 .

Discussion

The hyperaesthesia model chosen (formalin intra-articular injection) produces two periods of nociceptive response, with interpolation of a period of quiescence, due to an inhibition of nociceptive transmission around 5 to 10 minutes¹³. Thus, aiming to avoid evaluations in that period of quiescence, we decided that the first reassessment would occur 15 minutes after the completion of chemical stimulation, seeking a moment in which we could evaluate more reliably the nociception with respect to therapeutic procedures.

Considering that one of the most common treatments for lesions in the musculoskeletal system is the use of non-hormonal anti-inflammatory drugs, with their serious side effects (gastrointestinal ulcers, cardiovascular problems, etc.), other conservative treatments, such as low power laser and therapeutic ultrasound, are gaining importance as an option.^{9,18}

Alfredo *et al.*,¹ evaluating the use of laser associated with physical activity in patients with knee osteoarthritis, noted improvement in pain, range of motion and functionality, suggesting an anti-inflammatory activity and the endogenous modulation of pain through the action of serotonin as possible effects of the laser. The anti-inflammatory action may occur by changes in the cyclooxygenase pathway of arachidonic acid metabolism, besides the suppression of TNF- α , IL-1 β and hypoxia-inducing factor 1 α (HIF-1 α),^{2,3,18} other possible explanations are the peripheral release of β -endorphins¹⁹ and

an interruption of nerve conduction via thin fibers, due to the formation of axonal varicosities.²⁰ In the evaluation of the withdrawal threshold, we noted signs of reduction of nociception only when the stimulus was applied to the plantar surface, returning to baseline values at the first reassessment after treatment, and, in the comparison between groups in AV3, the threshold was significantly higher than placebo, but not different from the group treated with ultrasound. Although the comparison intragroup had not showed beneficial results in terms of stimulation on the knee, a significant increase in the threshold in both AV3 and AV4 versus placebo was noted. According to Jang and Lee,⁷ despite the controversy, the low power laser has proved to be effective in reducing joint pain. Thus, this is a useful tool, in view of the absence of side effects. These authors also point out that a major problem in the use of laser is to find the appropriate dose for each case. In the present study, fluency, wavelength and irradiance were used; previously, these parameters have proved to be able to reduce the joint pain in this experimental model.²¹

Tascioglu *et al.*⁹ evaluated the use of continuous (1 MHz, 2 W/cm², 5 min) or pulsed (2 W/cm² at 20% duty cycle) ultrasound for 10 treatment sessions in patients with knee osteoarthritis. These authors found that only the pulsed group had significant improvement in terms of pain and WOMAC index. They also reported that the analgesia produced by ultrasound can occur by thermal effects, which increase the pain threshold, and that non-thermal effects may increase the permeability of the cell membrane and, thus, the metabolic transport. In the present study, a significant reduction of the nociceptive situation in the animals treated with therapeutic ultrasound (in both places evaluated and in intra- and intergroup comparisons) was noted. However, despite our choice in favor of the continuous modality, we believe that the therapeutic effects were not due to hyperthermia. In this situation, it would be necessary to administer a higher dose, and the tissue temperature should increase between 40 and 45°C for at least 5 minutes.⁸ One possible explanation for the analgesic effect of non-thermal ultrasound is that the change produced in the cell membrane permeability can reduce the function of the sodium-potassium pump, thus hampering the nerve depolarization and leading to pain relief.²² Another explanation is the reduction of inducible nitric oxide synthase in the spinal cord, thereby reducing its activity in the processing of pain and inflammation.²³

According to Watson,⁸ the therapeutic effects of low power laser and therapeutic ultrasound are similar, and the biggest difference between these modalities lies in the fact that the absorption occurs in different types of tissues - therapeutic ultrasound is absorbed mainly in places with dense collagen tissue. This may have occurred in this study, since the joint capsule may have produced enhanced absorption and, therefore, most pronounced effects were obtained with the use of this modality when we evaluated the knee withdrawal threshold. In agreement with these aspects, Bakhtiary and Rashidy-Pour¹⁴ reported better results with ultrasound (1 MHz, 1.0 W/cm², pulsed 1:4), in the treatment of carpal tunnel syndrome of mild to moderate intensity, compared with low-level laser (9 J, 830 nm). Different results were obtained by Calis, Berberoglu and Calis²⁴ when comparing modalities (ultrasound 3 MHz, 1.5 W/cm², laser 904 nm, 1 J/cm²) in association with exercise and warm compresses

in patients with impingement syndrome. These authors found no benefits with any of the mentioned modalities.

Contrary to that observed in the present study, Charluz *et al.*²⁵ observed gains in patients with chronic low back pain with the use of low-level laser with respect to pain, while the ultrasound was more effective with respect to gains in lumbar extension. However, the application *modus* was different, in that the laser was applied in clusters of 808 nm, within an area of 100 cm², and the ultrasound was calibrated to 1 MHz, 1 W/cm² and application for 3 minutes. Rayegani *et al.*²⁶ also reported better results for the low power laser (880 nm, 39.7 J/cm²) compared to ultrasound (1.5 W/cm²) in patients with myofascial pain syndrome. The same was observed by Demir *et al.*²⁷ Comparing the techniques for experimental wound healing in mice, these authors observed better effects for laser (904 nm, 1 J/cm², while ultrasound was calibrated to 0.5 W/cm²) in the inflammatory and proliferation phases.

The results of this study show that in the group of combined techniques, during the assessment of nociception in the planar region an increase in the withdrawal threshold was noted, with restoration to the previous levels after one hour of hyperaesthesia induction, but there was no improvement regarding the evaluation of threshold on the knee. Only in the comparison with placebo in AV4 an increase in the threshold was observed. Thus, it is inferred that when used alone, the therapies showed better results than the combination of the two techniques. This fact was also observed by Gum *et al.*²⁸ In assessing the effects of the combination of low level laser (904 nm, 1 J/cm²) with therapeutic ultrasound (1 MHz, 0.5 W/cm²) in tenotomized rabbits, these authors observed an increase in collagen synthesis, but only with trends of improvement in the biomechanical properties of tendons (which was observed in previous studies, with the exclusive application of each of these modalities). Demir *et al.*²⁹ noted improvement in the healing of injured tendons of rats subjected to laser treatment (904 nm, 6 mW, 1 J/cm²), ultrasound (1 MHz, 0.5 W/cm²) or with the combination of these techniques, without any cumulative effects of this association.

Thus, it appears that a combination of techniques does not result in benefit, considering the results presented here and those of our brief review of the literature.

Conclusion

It can be concluded that both treatment modalities had anti-nociceptive effects, and the therapeutic ultrasound was superior versus laser and the combination of techniques, namely, the summation of effects did not occur with the use of this latter strategy.

Conflicts of interest

The authors declare no conflicts of interest.

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